Zoonotic Pathogens in Wildlife Traded in Markets for Human Consumption, Laos

Pruksa Nawtaisong,[1] Matthew T. Robinson,[1] Khongsy Khammavong, Phonesavanh Milavong, Audrey Rachlin,[2] Sabine Dittrich,[3] Audrey Dubot-Pérès, Malavanh Vongsouvath, Paul F. Horwood,[4] Philippe Dussart,[5] Watthana Theppangna, Bounlom Douangngeum, Amanda E. Fine, Mathieu Pruvot,[6] Paul N. Newton[6]

We tested animals from wildlife trade sites in Laos for the presence of zoonotic pathogens. *Leptospira* spp. were the most frequently detected infectious agents, found in 20.1% of animals. *Rickettsia typhi* and *R. felis* were also detected. These findings suggest a substantial risk for exposure through handling and consumption of wild animal meat.

Consumption of wildlife meat drives emerging infectious diseases (*1*), often amplified by human encroachment into natural areas and changes in land use. Wildlife trade and consumption have been responsible for outbreaks of diseases such as HIV-1 (*2*), Ebola (*3*), and monkeypox (*4*) and possibly for the coronavirus disease pandemic (*5*). Wildlife markets bring diverse species into contact, usually in dense and unsanitary conditions, enabling mixing, amplification, and transmission of pathogens among species, including humans (*6*). Small mammals host diverse pathogenic bacteria and viruses (*7*), but little investigation of endemic bacteria transmission has occurred. Determining pathogens present in traded wildlife is vital to guide appropriate measures to combat zoonotic diseases and document societal and environmental costs of wildlife trade.

The Study

During December 2014–September 2017, we collected samples from 9 wildlife trade hotspots (*8*) and 2 roadside stalls (hereafter all referred to as trade sites) in Laos (Figure; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/4/21-0249-App1.pdf). In addition, 3 Provincial Offices of Forest Inspection (POFI) collected samples from wildlife confiscated in markets by law enforcement. After identifying wildlife at trade sites (*9*), we asked vendors for permission to sample their animals. Depending on whether the animal was alive, dead, or butchered, we collected urogenital swabs, urine and blood samples, and kidney, liver, and spleen tissue samples (Appendix Table 2).

We extracted nucleic acid using QIAamp Viral RNA Mini Kits (QIAGEN, https://www.qiagen.com) with modifications (Appendix). We conducted PCRs targeting *Leptospira* spp., *Rickettsia* spp., *Orientia tsutsugamushi*, Anaplasmataceae, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Coxiella burnetti*, flaviviruses, hantavirus, dengue virus, Zika virus, and universal bacterial 16S rRNA (Appendix Table 3). Where necessary, PCR products were sequenced (Macrogen Inc., https://www.macrogen.

Author affiliations: Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Mahosot Hospital, Vientiane, Laos (P. Nawtaisong, M.T. Robinson, A. Rachlin, S. Dittrich, A. Dubot-Pérès, M. Vongsouvath, P.N. Newton); University of Oxford Centre for Tropical Medicine and Global Health, Oxford, UK (M.T. Robinson, S. Dittrich, A. Dubot-Pérès, P.N. Newton); Wildlife Conservation Society, Bronx, New York, USA (K. Khammavong, P. Milavong, A.E. Fine, M. Pruvot); Unité des Virus Émergents (UVE) Aix-Marseille Univ-IRD 190-Inserm 1207), Marseille, France (A. Dubot-Pérès); Institut Pasteur du Cambodge, Phnom Penh, Cambodia (P.F. Horwood, P. Dussart); National Animal Health Laboratory, Ministry of Agriculture, Vientiane (W. Theppangna, B. Douangngeum); University of Calgary, Calgary, Alberta, Canada (M. Pruvot)

DOI: https://doi.org/10.3201/eid2804.210249

[1]These first authors contributed equally to this article.

[2]Current affiliation: Menzies School of Health Research, Darwin, Northern Territory, Australia.[3]Current affiliation: Campus Biotech, Geneva, Switzerland; University of Oxford Centre for Tropical Medicine and Global Health, Oxford, UK.[4]Current affiliation: James Cook University, Townsville, Queensland, Australia.

[5]Current affiliation: Institut Pasteur de Madagascar, Antananarivo, Madagascar.

[6]These authors contributed equally to this article.

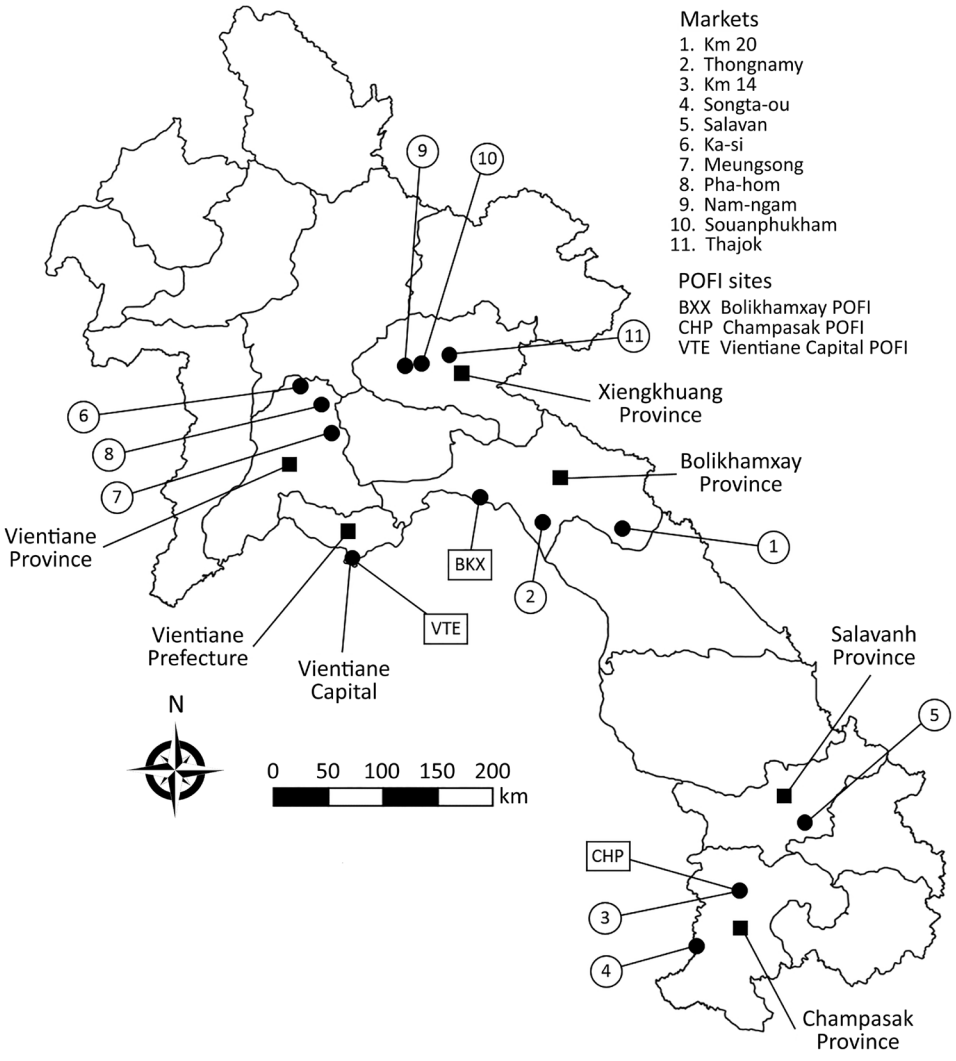

Figure. Wildlife trade sites and POFI sites (black circles) where wildlife samples were collected for study of zoonotic pathogens in wildlife traded in markets for human consumption, Laos. Provinces are labeled with black squares. POFI, Provincial Office of Forestry Inspection.

com) and compared against GenBank through blastn (https://blast.ncbi.nlm.nih.gov). We performed descriptive, univariate, and multivariate analyses by using R version 3.6.2 (https://www.r-project.org). We assessed the effect of the wild meat processing status (alive, fresh, or frozen) on the risk for *Leptospira* detection by using a mixed effects logistic regression with species as random effect. Statistical significance was set at $\alpha = 0.05$ (Appendix).

We collected 717 samples from 359 animals (trade sites: 461 samples from 324 animals; POFI: 256 samples from 35 animals); animals sampled were from ≥37 identifiable vertebrate species from 12 families (Appendix Table 4). Most were Sciuridae squirrels (73.0%, 262/359) and represented 16 species, most frequently Pallas's squirrel (*Callosciurus erythraeus*) (20.3%, 73/359). From trade sites, 69 animals (21.3%, 95% CI 17.0%–26.2%) had ≥1 samples positive for ≥1 pathogens in 10 of 11 sites (90.9%, 95% CI 57.1%–99.5%) (Appendix Table 5). Of 324 animals tested, 65 (20.1%, 95% CI 15.9%–24.9%) were positive for *Leptospira* spp.; 4/41 were positive for *Rickettsia* spp. (9.8%, 95% CI 3.2%–24.1%), 0 for *O. tsutsugamushi* (0%, 95% CI 0%–10.7%), and 2 for Anaplasmataceae (4.9%, 95% CI 0.8%–17.8%) (Table 1). Positivity was higher among animals collected by POFI; 25/35 (71.4%) animals tested positive for ≥1 pathogens. Of those, 9 were positive for *Leptospira* spp. (25.7%, 95% CI 13.1%–43.6%), 20 for *Rickettsia* spp. (57.1%, 95% CI 39.5%–73.2%), 2 for *O. tsutsugamushi* (5.7%, 95% CI 1.0%–20.5%), and 6 for Anaplasmataceae (17.1%, 95% CI 7.2%–34.3%) (Table 2). Sequencing identified *R. typhi*, *R. felis*, *R. conorii*, an *Anaplasma* species (either *A. centrale*, *A. capra*, or *A. marginale*), *A. platys*, *A. bovis*, *A. phagocytophilum*, *Ehrlichia chaffeensis*, *Lactococcus garvieae*, and *Kurthia populi* (Tables 1, 2). No

Table 1. Zoonotic pathogens detected and animal species and sample types that tested positive in wildlife collected from trade sites, Laos*

Organism	No. positive/no. tested				Sequencing identity match, %†
	Animals	Species	Samples	Sample types	
Leptospira spp.	65/324	*Callosciurus finlaysonii* squirrel, 13/28 *C. erythraeus* squirrel, 8/56 *Paradoxurus hermaphroditus* civet, 10/22 *C. inornatus* squirrel, 7/34 *Dremomys rufigenis* squirrel, 5/35 *Menetes berdmorei* ground squirrel, 4/29 *Rhizomys pruinosus* rat, 3/21 *Arctogalidia trivirgata* civet, 2/2 *Petaurista philippensis* flying squirrel, 1/9 *Atherurus macrourus* porcupine, 1/1 *Belomys pearsonii* flying squirrel, 1/12 *Eonycteris spelaea* bat, 1/3 *Hylopetes alboniger* flying squirrel, 1/5 *H. phayrei* flying squirrel, 1/9 *H. spadiceus* flying squirrel, 1/2 *Muntiacus muntjak* deer, 1/1 *Paguma larvata* civet, 1/2 *Prionailurus bengalensis* cat, 1/3 Rhizomys *sumatrensis* rat, 1/6 *Tupaia belangeri* treeshrew, 1/3 Unknown Sciuridae squirrel, 1/2	72/461	URO, 58/312 SPL, 1/3 KID, 2/6 LIV, 1/40 BLD, 9/85 URI, 1/15	NA
Rickettsia spp.	1/41	*P. philippensis* flying squirrel, 1/2	1/68	LIV, 1/40	NA
Rickettsia felis†	2/41	*D. rufigenis* squirrel, 1/11 *P. hermaphroditus* civet, 1/6	2/68	LIV, 2/40	98–100
R. typhi†	1/41	*D. rufigenis* squirrel, 1/11	1/68	LIV, 1/40	93
Anaplasma platys†	1/41	*P. hermaphroditus* civet, 1/6	1/68	KID, 1/6	98
A. centrale	1/41	*M. muntjak* deer, 1/1	5/68	KID, 1/6	98.8–99.6 (*A. centrale*)
A. capra				LIV, 3/40	98.8–99.6 (*A. capra*)
A. marginale†				SPL, 1/3	98.8 (*A. marginale*)

*BLD, blood; KID, kidney; LIV, liver; NA, not applicable; SPL, spleen; URI, urine; URO, urogenital swab.
†Organism identified by sequencing of PCR products and identity match given in the right-hand column. All nucleotide sequences were submitted to GenBank under accession nos. MW407963–MW407984 and MW411434–MW411439.

samples were positive for *C. burnetii* (0/76), flaviviruses (0/359), dengue virus (0/359), or Zika virus (0/358).

Among species for which >10 individual animals were sampled in trade sites, 2 had particularly high proportions of *Leptospira* spp.–positive specimens: the variable squirrel (*Callosciurus finlaysonii*) (13/28; 46.4% 95% CI 28.0%–65.8%) and the common palm civet (*Paradoxurus hermaphroditus)* (10/22; 45.5%, 95% CI 25.2%–67.3%). *Leptospira* spp.–positivity was higher in dry (50/195; 25.6%, 95% CI 19.8%–32.5%) than wet season (15/129; 11.6%, 95% CI 6.9%–18.8%) (χ^2 = 8.7; p = 0.003). Data disaggregation by species and province suggested that observed seasonality was driven by results in common palm civets and variable squirrels in Champasak Province. No association was detected between the probability of an animal testing positive for *Leptospira* and the animal being alive (3/22; 14%, 95% CI 3.6%–36%), freshly dead (58/293; 20%, 95% CI 16%–25%; p = 0.6), or frozen (4/9; 44%, 95% CI 15%–77%; p = 0.1). In a subset of *Leptospira* spp.–positive animals with multiple samples, 75% (18/24; 95% CI 53%–89%) of urogenital swab samples and 50% (9/18; 95% CI 29%–71%) of blood samples were positive (p = 0.11 by Fisher exact test). *Rickettsia* spp. were detected exclusively in solid organs (liver, kidney, and spleen).

Zoonotic pathogens were nearly ubiquitous across sites; 10/11 sites yielded ≥1 pathogens. Squirrels are frequently traded in Lao markets (*8*) and had the greatest pathogen diversity in this study. *Leptospira* spp. was identified most frequently, found in 20.1% of animals (>45% in variable squirrels and common palm civets). Variable squirrels are commonly traded, often in batches of 2 to 3 squirrels (*8*); hence, on average, someone purchasing 3 variable squirrels would have an 83% likelihood of buying ≥1 infected squirrel ($p = 1 - (1 - \text{prevalence})^3 = 1 - 0.55^3 = 0.83$). The higher risk for *Leptospira* detection in the dry season is at odds with the typically described correlation of transmission with precipitation and flooding (*10*), suggesting that much remains to be understood of *Leptospira* ecology. Other

studies have shown higher prevalence in rats (*11*), and although we are confident of the results from trade sites, storage of animals from POFI sites might have resulted in cross-contamination, which warrants cautious interpretation of results in this subset. Among *Leptospira* spp.-positive animals, detection was more likely in urogenital swab samples, highlighting the risk for transmission through infected urine (*10*). Although reservoir rodents are characterized by chronic renal infections, septicemia occurs during initial infection (*10*), and the high proportion of positive blood samples indicates a public health risk in relation to the consumption of uncooked or undercooked meat, organs, and blood. The PCR used to detect leptospires is specific for pathogenic and intermediate species (Appendix Table 3), but we could not confirm their human pathogenicity. The high volume of squirrel trade combined with high infection frequency suggests a high risk for exposure among wildlife consumers. Because leptospirosis is a key cause of fever in rural Laos (*12*), further work is needed to learn more about the relevance of contact with wildlife through trade and consumption.

The Rickettsiales species identified here are known to cause human infections in Laos (*13*). *R. typhi* causes murine typhus, a major underrecognized cause of fever (*13*). *O. tsutsugamushi* is responsible for up to 23% of fever (*14*), and although commonly associated with ground-dwelling rodents, the vectors (*Leptotrombidium* mites) parasitize squirrels (*15*), and *O. tsutsugamushi* has been isolated from *Callosciurus notatus* squirrels in Malaysia (*16*). Other bacteria identified are reviewed elsewhere (Appendix Table 6).

Although many of the human pathogens identified are transmitted by arthropod vectors, we found few arthropods in the wildlife sampled, probably because vectors leave animals quickly after animal death (*17*). Therefore, because most market vendors sell dead animals obtained from hunters or intermediaries (*8*), vendors are less likely to be exposed to disease vectors, and hunters are possibly at greater risk than market vendors or consumers. *O. tsutsugamushi* and *R. typhi* can cause infections through aerosol exposure, bites from infected animals, and needlestick injuries (*18*), but whether such routes of infection occur at trade sites is unclear. The frequent occurrence of *Leptospira*, which can be transmitted by direct contact with abraded skin and mucous membranes, may pose health risks to hunters, vendors, and consumers.

Table 2. Zoonotic pathogens detected and animal species and sample types that tested positive in wildlife collected from POFI sites*

Organism	No. positive/no. tested				Sequencing identity match, %†
	Animals	Species	Samples	Sample types	
Leptospira spp.	9/35	*Callosciurus finlaysonii* squirrel, 1/1 *Callosciurus erythraeus* squirrel, 4/17 *Callosciurus inornatus* squirrel, 2/6 *Petaurista philippensis* flying squirrel, 1/5 *Catopuma temminckii* cat, 1/1	46/256	SPL, 17/69 KID, 14/91 LIV, 14/92 BLD, 1/3	NA
Orientia tsutsugamushi	2/34	*C. erythraeus* squirrel, 2/17	2/252	SPL, 2/252	NA
Rickettsia spp.	12/35	*C. erythraeus* squirrel, 5/17 *P. philippensis* flying squirrel, 2/5 *C. inornatus* squirrel, 2/6 *Paradoxurus hermaphroditus* civet, 1/2 *Catopuma temminckii* cat, 1/1 *Ratufa bicolor* squirrel, 1/1	70/252	LIV, 30/92 KID, 25/91 SPL, 15/69	NA
Rickettsia conorii†	1/35	*P. philippensis* flying squirrel, 1/5	1/252	LIV, 1/92	99
R. felis†	1/35	*C. erythraeus* squirrel, 1/17	2/252	LIV, 1/92 SPL, 1/69	98
R. typhi	6/35	*C. erythraeus* squirrel, 6/17	7/252	KID, 4/91 LIV, 2/92 SPL, 1/69	NA
Anaplasmataceae	1/34	*C. erythraeus* squirrel, 1/17	3/252	KID, 2/91 SPL, 1/69	NA
Anaplasma bovis†	1/34	*C. erythraeus* squirrel, 1/17	7/252	KID, 1/91 LIV, 3/92 SPL, 3/69	99.7–100
A. phagocytophilum†	2/34	*Catopuma temminckii* cat, 1/1 *P. philippensis* flying squirrel, 1/4	4/252	KID, 2/91 SPL, 2/69	98–99
Ehrlichia spp./*E. chaffeensis*†	1/34	Unknown Muridae rat, 1/1	1/252	SPL, 1/69	97 (*Ehrlichia* spp.) 97 (*E. chaffeensis*)
Kurthia populi†	1/34	*C. erythraeus* squirrel, 1/17	1/252	LIV, 1/92	98
Lactococcus garvieae†	1/34	*C. erythraeus* squirrel, 1/17	1/252	SPL, 1/69	99

*BLD, blood; KID, kidney; LIV, liver; NA, not applicable; POFI, Provincial Office of Forestry Inspection; SPL, spleen; URI, urine; URO, urogenital swab.
†Organism identified by sequencing of PCR products and identity match given in righthand column. All nucleotide sequences were submitted to GenBank under accession nos. MW407963–MW407984 and MW411434–MW411439.

Acknowledgments

We thank the market managers and vendors for their participation and the Provincial Offices of Forest Inspection and Provincial Livestock and Fishery Section for their assistance. We also thank the Director and staff of Mahosot Hospital and the Microbiology Laboratory, the Wildlife Conservation Society Lao PDR Program, and the Minister of Health of the Lao PDR for their support. We are very grateful to the late Rattanaphone Phetsouvanh and Manivanh Vongsouvath and Mayfong Mayxay for their support for this work.

Study protocols were reviewed and authorized by the Wildlife Conservation Society's Institutional Animal Care and Use Committee under permit 15:04, and by the National Animal Health Laboratory, Ministry of Agriculture and Forestry, Laos.

This study was funded by the European Union under the INNOVATE program and the LACANET project (DCIASIE/2013/315-151). This work was funded in whole or in part by the Wellcome Trust (grant number: 220211). M.T.R. and P.N.N. are funded by Wellcome.

About the Author

Dr. Nawtaisong is a postdoctoral researcher specializing in molecular microbiology of zoonotic pathogens and previously worked at the Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU).

References

1. Karesh WB, Cook RA, Bennett EL, Newcomb J. Wildlife trade and global disease emergence. Emerg Infect Dis. 2005;11:1000–2. https://doi.org/10.3201/eid1107.050194
2. Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: scientific and public health implications. Science. 2000;287:607–14. https://doi.org/10.1126/science.287.5453.607
3. Leroy EM, Rouquet P, Formenty P, Souquière S, Kilbourne A, Froment JM, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science. 2004;303:387–90. https://doi.org/10.1126/science.1092528
4. Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV, et al. The detection of monkeypox in humans in the Western Hemisphere. N Engl J Med. 2004;350:342–50. https://doi.org/10.1056/NEJMoa032299
5. Li X, Giorgi EE, Marichannegowda MH, Foley B, Xiao C, Kong X-P, et al. Emergence of SARS-CoV-2 through recombination and strong purifying selection. Sci Adv. 2020;6:eabb9153. https://doi.org/10.1126/sciadv.abb9153
6. Huong NQ, Nga NTT, Long NV, Luu BD, Latinne A, Pruvot M, et al. Coronavirus testing indicates transmission risk increases along wildlife supply chains for human consumption in Viet Nam, 2013-2014. PLoS One. 2020; 15:e0237129. https://doi.org/10.1371/journal.pone.0237129
7. Mills JN, Childs JE. Ecologic studies of rodent reservoirs: their relevance for human health. Emerg Infect Dis. 1998;4:529–37. https://doi.org/10.3201/eid0404.980403
8. Pruvot M, Khammavong K, Milavong P, Philavong C, Reinharz D, Mayxay M, et al. Toward a quantification of risks at the nexus of conservation and health: the case of bushmeat markets in Lao PDR. Sci Total Environ.2019; 676:732–45. https://doi.org/10.1016/j.scitotenv.2019.04.266
9. Francis CM. A field guide to the mammals of southeast Asia. London: New Holland; 2008.
10. Levett PN. Leptospirosis. Clin Microbiol Rev. 2001;14:296–326. https://doi.org/10.1128/CMR.14.2.296-326.2001
11. Blasdell KR, Morand S, Perera D, Firth C. Association of rodent-borne *Leptospira* spp. with urban environments in Malaysian Borneo. PLoS Negl Trop Dis. 2019;13:e0007141. https://doi.org/10.1371/journal.pntd.0007141
12. Mayxay M, Castonguay-Vanier J, Chansamouth V, Dubot-Pérès A, Paris DH, Phetsouvanh R, et al. Causes of non-malarial fever in Laos: a prospective study. Lancet Glob Health. 2013;1:e46–54. https://doi.org/10.1016/S2214-109X(13)70008-1
13. Phongmany S, Rolain J-M, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B, et al. Rickettsial infections and fever, Vientiane, Laos. Emerg Infect Dis. 2006;12:256–62. https://doi.org/10.3201/eid1202.050900
14. Kurup A, Issac A, Loh JP, Lee TB, Chua R, Bist P, et al. Scrub typhus with sepsis and acute respiratory distress syndrome. J Clin Microbiol. 2013;51:2787–90. https://doi.org/10.1128/JCM.00463-13
15. Choe S, Lee D, Park H, Jeon HK, Lee Y, Na KJ, et al. *Catenotaenia dendritica* (Cestoda: Catenotaeniidae) and three ectoparasite species in the red squirrel, *Sciurus vulgaris*, from Cheongju, Korea. Korean J Parasitol. 2016;54:509–18. https://doi.org/10.3347/kjp.2016.54.4.509
16. Walker JS, Gan E, Muul I, Muul I; Chan Teik Chye. Involvement of small mammals in the transmission of scrub typhus in Malaysia: isolation and serological evidence. Trans R Soc Trop Med Hyg. 1973;67:838–45. https://doi.org/10.1016/0035-9203(73)90012-6
17. Feldhamer GA, Drickamer LC, Vessey SH, Merritt JF, Krajewski C. Mammalogy: adaptation, diversity, ecology. Baltimore (MD): Johns Hopkins University Press; 2007.
18. Blacksell SD, Robinson MT, Newton PN, Day NPJ. Laboratory-acquired scrub typhus and murine typhus infections: the argument for a risk-based approach to biosafety requirements for *Orientia tsutsugamushi* and *Rickettsia typhi* laboratory activities. Clin Infect Dis. 2019;68:1413–9. https://doi.org/10.1093/cid/ciy675

Address for correspondence: Matthew Robinson, LOMWRU, Microbiology Laboratory, Mahosot Hospital, Quai Fa Ngum, Vientiane City, Vientiane 01000, Laos; email: matthew.r@tropmedres.ac

Article DOI: https://doi.org/10.3201/eid2804.210249

Zoonotic Pathogens in Wildlife Traded in Markets for Human Consumption, Laos

Appendix

Supplementary Methods

Data Collection

Identification of animals was done to species level when possible by using an established regional field guide (*1*). Basic information, such as the status of the animal (live or dead) and storage status (frozen or fresh), was recorded.

Extraction of Nucleic Acids

We extracted samples by using the QIAamp Viral RNA Mini Kits (QIAGEN, https://www.qiagen.com). The manufacturer's protocol was modified following manufacturer's recommendations (*2*) and DNA and RNA were extracted simultaneously. Urogenital swabs were first centrifuged to release cells from the swabs into the supernatant, which was then transferred into new tubes. For blood and urine, 200 µL of sample was used. Buffer AL (200 µL) and Proteinase K (20 µL) were added to the samples and incubated at 56°C for 30 minutes. For liver, spleen, and kidney tissue, 0.025 g of tissue was transferred to a tube containing glass beads. Buffer ATL (200 µL) and Proteinase K (20 µL) were added and the sample vortexed for 30 minutes, then incubated as described previously. To check for the presence of PCR inhibitors, 10 µL of T4/MS2 phage solution (*3*) was added after cell lysis. The extraction process was continued as per manufacturer's protocol with minor adjustments: 800 µL AVL/carrier RNA buffer, 200 µL ethanol, and 500 µL AW1 and AW2. Total nucleic acid was eluted in 100 µL Buffer AVE.

Statistical Analysis

Descriptive, univariate, and multivariate analyses were done using R version 3.6.2 (https://www.r-project.org). Confidence interval around prevalence estimates and other proportions used binomial confidence intervals. The effect of season on *Leptospira* prevalence

was initially assessed by a χ^2 test. To further test the season effect in different provinces, we used a logistic regression with season, province, and their interaction as explanatory variables. The effect of the wild meat preservation method on the risk for *Leptospira* detection was assessed by using a mixed effects logistic regression with species as random effect, using the R package *lme4*. In a subset of *Leptospira* spp.-positive animals for which multiple sample types were available for testing (i.e., animals have multiple sample types, and $\geq$1 of them is positive for *Leptospira*), we compared the proportion of positive results in genital swab samples and blood samples by using a Fisher exact test.

Finally, to further demonstrate the significance of the high prevalence of *Leptospira* spp. found in squirrels, we estimated the probability of a consumer being exposed to *Leptospira* in the simple and common scenario where a consumer purchases 3 variable squirrels. With a known prevalence p of *Leptospira* spp. in variable squirrels, the probability of a consumer to purchase $\geq$1 infected animal is one minus the probability to purchase no infected animals. Assuming that the infection status of individual squirrels on a stall is independent, this is P(purchasing $\geq$1 infected squirrel among) = $1-(1-p)^3$.

References

1. Francis CM. A field guide to the mammals of southeast Asia. London: New Holland; 2008.

2. QIAGEN. QIAamp DNA mini and blood mini handbook. 5th ed. 2016. pp 72.

3. Ninove L, Nougairede A, Gazin C, Thirion L, Delogu I, Zandotti C, et al. RNA and DNA bacteriophages as molecular diagnosis controls in clinical virology: a comprehensive study of more than 45,000 routine PCR tests. PLoS One. 2011;6:e16142. PubMed https://doi.org/10.1371/journal.pone.0016142

4. Jiang J, Chan TC, Temenak JJ, Dasch GA, Ching WM, Richards AL. Development of a quantitative real-time polymerase chain reaction assay specific for *Orientia tsutsugamushi*. Am J Trop Med Hyg. 2004;70:351–6. PubMed https://doi.org/10.4269/ajtmh.2004.70.351

5. Jiang J, Stromdahl EY, Richards AL. Detection of *Rickettsia parkeri* and *Candidatus* Rickettsia andeanae in *Amblyomma maculatum* Gulf Coast ticks collected from humans in the United States. Vector Borne Zoonotic Dis. 2012;12:175–82. PubMed https://doi.org/10.1089/vbz.2011.0614

6. Slack A, Symonds M, Dohnt M, Harris C, Brookes D, Smythe L. Evaluation of a modified Taqman assay detecting pathogenic *Leptospira* spp. against culture and *Leptospira*-specific IgM enzyme-linked immunosorbent assay in a clinical environment. Diagn Microbiol Infect Dis. 2007;57:361–6. PubMed https://doi.org/10.1016/j.diagmicrobio.2006.10.004

7. Smythe LD, Smith IL, Smith GA, Dohnt MF, Symonds ML, Barnett LJ, et al. A quantitative PCR (TaqMan) assay for pathogenic *Leptospira* spp. BMC Infect Dis. 2002;2:13. PubMed https://doi.org/10.1186/1471-2334-2-13

8. Thaipadungpanit J, Chierakul W, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Boonslip S, et al. Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and lipL32 genes for human leptospirosis in Thailand: a case-control study [Erratum in: PLoS One. 2011;6]. PLoS One. 2011;6:e16236. PubMed https://doi.org/10.1371/journal.pone.0016236

9. Parola P, Roux V, Camicas JL, Baradji I, Brouqui P, Raoult D. Detection of ehrlichiae in African ticks by polymerase chain reaction. Trans R Soc Trop Med Hyg. 2000;94:707–8. PubMed https://doi.org/10.1016/S0035-9203(00)90243-8

10. Loftis AD, Massung RF, Levin ML. Quantitative real-time PCR assay for detection of *Ehrlichia chaffeensis.* J Clin Microbiol. 2003;41:3870–2. PubMed https://doi.org/10.1128/JCM.41.8.3870-3872.2003

11. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of *anaplasma phagocytophilum* and *Borrelia burgdorferi.* J Clin Microbiol. 2004;42:3164–8. PubMed https://doi.org/10.1128/JCM.42.7.3164-3168.2004

12. Tilburg JJ, Horrevorts AM, Peeters MF, Klaassen CH, Rossen JW. Identification by genotyping of a commercial antigen preparation as the source of a laboratory contamination with *Coxiella burnetii* and as an unexpected rich source of control DNA. J Clin Microbiol. 2011;49:383–4. PubMed https://doi.org/10.1128/JCM.01491-10

13. Moureau G, Temmam S, Gonzalez JP, Charrel RN, Grard G, de Lamballerie X. A real-time RT-PCR method for the universal detection and identification of flaviviruses. Vector Borne Zoonotic Dis. 2007;7:467–77. PubMed https://doi.org/10.1089/vbz.2007.0206

14. Leparc-Goffart I, Baragatti M, Temmam S, Tuiskunen A, Moureau G, Charrel R, et al. Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses. J Clin Virol. 2009;45:61–6. PubMed https://doi.org/10.1016/j.jcv.2009.02.010

15. Mohamed N, Nilsson E, Johansson P, Klingström J, Evander M, Ahlm C, et al. Development and evaluation of a broad reacting SYBR-green based quantitative real-time PCR for the detection of different hantaviruses. J Clin Virol. 2013;56:280–5. PubMed https://doi.org/10.1016/j.jcv.2012.12.001

16. Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 1989;17:7843–53. PubMed https://doi.org/10.1093/nar/17.19.7843

17. Cleenwerck I, Camu N, Engelbeen K, De Winter T, Vandemeulebroecke K, De Vos P, et al. *Acetobacter ghanensis* sp. nov., a novel acetic acid bacterium isolated from traditional heap fermentations of Ghanaian cocoa beans. Int J Syst Evol Microbiol. 2007;57:1647–52. PubMed https://doi.org/10.1099/ijs.0.64840-0

18. Ninove L, Nougairede A, Gazin C, Thirion L, Delogu I, Zandotti C, et al. RNA and DNA bacteriophages as molecular diagnosis controls in clinical virology: a comprehensive study of more than 45,000 routine PCR tests. PLoS One. 2011;6:e16142. PubMed https://doi.org/10.1371/journal.pone.0016142

19. Phongmany S, Rolain J-M, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B, et al. Rickettsial infections and fever, Vientiane, Laos. Emerg Infect Dis. 2006;12:256–62. PubMed https://doi.org/10.3201/eid1202.050900

20. Angelakis E, Mediannikov O, Parola P, Raoult D. *Rickettsia felis*: the complex journey of an emergent human pathogen. Trends Parasitol. 2016;32:554–64. PubMed https://doi.org/10.1016/j.pt.2016.04.009

21. Kurup A, Issac A, Loh JP, Lee TB, Chua R, Bist P, et al. Scrub typhus with sepsis and acute respiratory distress syndrome. J Clin Microbiol. 2013;51:2787–90. PubMed https://doi.org/10.1128/JCM.00463-13

22. Choe S, Lee D, Park H, Jeon HK, Lee Y, Na KJ, et al. *Catenotaenia dendritica* (Cestoda: Catenotaeniidae) and three ectoparasite species in the red squirrel, *Sciurus vulgaris*, from Cheongju, Korea. Korean J Parasitol. 2016;54:509–18. PubMed https://doi.org/10.3347/kjp.2016.54.4.509

23. Walker JS, Gan E, Muul I, Muul I; Chan Teik Chye. Involvement of small mammals in the transmission of scrub typhus in Malaysia: isolation and serological evidence. Trans R Soc Trop Med Hyg. 1973;67:838–45. PubMed https://doi.org/10.1016/0035-9203(73)90012-6

24. Atif FA. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. Parasitol Res. 2015;114:3941–57. PubMed https://doi.org/10.1007/s00436-015-4698-2

25. Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, et al. Intravascular persistence of *Anaplasma platys, Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. Parasit Vectors. 2014;7:298. PubMed https://doi.org/10.1186/1756-3305-7-298

26. Li H, Zheng Y-C, Ma L, Jia N, Jiang B-G, Jiang R-R, et al. Human infection with a novel tick-borne *Anaplasma* species in China: a surveillance study. Lancet Infect Dis. 2015;15:663–70. PubMed https://doi.org/10.1016/S1473-3099(15)70051-4

27. Yang J, Liu Z, Niu Q, Mukhtar MU, Guan G, Liu G, et al. A novel genotype of "*Anaplasma capra*" in wildlife and its phylogenetic relationship with the human genotypes. Emerg Microbes Infect. 2018;7:210. PubMed https://doi.org/10.1038/s41426-018-0212-0

28. Costa SC, de Magalhães VC, de Oliveira UV, Carvalho FS, de Almeida CP, Machado RZ, et al. Transplacental transmission of bovine tick-borne pathogens: Frequency, co-infections and fatal neonatal anaplasmosis in a region of enzootic stability in the northeast of Brazil. Ticks Tick Borne Dis. 2016;7:270–5. PubMed https://doi.org/10.1016/j.ttbdis.2015.11.001

29. Howden KJ, Geale DW, Paré J, Golsteyn-Thomas EJ, Gajadhar AA. An update on bovine anaplasmosis (*Anaplasma marginale*) in Canada. Can Vet J. 2010;51:837–40. PubMed

30. Dong T, Qu Z, Zhang L. Detection of *A. phagocytophilum* and *E. chaffeensis* in patient and mouse blood and ticks by a duplex real-time PCR assay. PLoS One. 2013;8:e74796. PubMed https://doi.org/10.1371/journal.pone.0074796

31. Chan JF, Woo PC, Teng JL, Lau SK, Leung SS, Tam FC, et al. Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. Infection. 2011;39:259–64. PubMed https://doi.org/10.1007/s15010-011-0094-8

32. Ongrádi J, Stercz B, Kövesdi V, Nagy K, Chatlynne L. Isolation of *Kurthia gibsonii* from non-gonorrheal urethritis: implications for the pathomechanism upon surveying the literature. Acta Microbiol Immunol Hung. 2014;61:79–87. PubMed https://doi.org/10.1556/amicr.61.2014.1.8

Appendix Table 1. Location and date of wildlife sampled from markets, Laos*

ID	Province	Site	Site classification	Season	Date of visit	No. animals sampled	No. animals sampled (% of total)	No. animals positive by PCR (% of animals from site)
1	Bolikhamxay	Km 20 market	Trade	Dry	2016 Jan 22	8	49 (13.7)	5 (10.2)
				Dry	2016 Jan 23	3		
				Dry	2016 Jan 24	6		
				Dry	2016 Feb 26	3		
				Dry	2016 Feb 27	6		
				Dry	2016 Feb 28	10		
				Wet	2015 Aug 20	6		
				Wet	2015 Aug 21	5		
				Wet	2017 Sep 19	2		
2	Bolikhamxay	Thongnamy	Trade	Dry	2016 Jan 25	9	23 (6.4)	8 (34.8)
				Dry	2016 Jan 26	4		
				Dry	2016 Mar 1	3		
				Wet	2016 May 4	2		
				Wet	2016 May 5	5		
3	Champasak	Km 14 market	Trade	Dry	2015 Dec 17	4	49 (13.7)	15 (30.6)
				Dry	2015 Dec 18	5		
				Dry	2015 Dec 19	5		
				Dry	2016 Feb 5	5		
				Dry	2016 Feb 6	1		
				Dry	2016 Feb 7	6		
				Dry	2016 Feb 9	7		
				Wet	2015 May 23	7		
				Wet	2015 May 24	4		
				Wet	2015 May 25	4		
				Wet	2015 May 26	1		
4	Champasak	Songta-ou	Trade	Dry	2015 Nov 2	1	55 (15.3)	12 (21.8)
				Dry	2015 Dec 16	11		
				Dry	2015 Dec 21	8		
				Dry	2016 Feb 8	10		
				Dry	2016 Feb 10	9		
				Wet	2015 May 20	9		
				Wet	2015 May 22	7		
5	Saravanh	Salavan Market	Trade	Dry	2015 Dec 23	5	44 (12.3)	9 (20.5)
				Dry	2015 Dec 24	4		
				Dry	2015 Dec 25	2		
				Dry	2016 Feb 11	6		
				Dry	2016 Feb 12	2		
				Dry	2016 Feb 13	3		
				Dry	2016 Feb 14	3		
				Dry	2016 Apr 30	5		
				Wet	2015 May 28	7		
				Wet	2015 May 29	4		
				Wet	2016 May 1	3		
6	Vientiane	Ka-si	Trade	Dry	2016 Mar 15	10	40 (11.1)	6 (15.0)
				Dry	2016 Mar 16	6		
				Wet	2015 Aug 8	5		

ID	Province	Site	Site classification	Season	Date of visit	No. animals sampled	No. animals sampled (% of total)	No. animals positive by PCR (% of animals from site)
				Wet	2015 Aug 9	2		
				Wet	2015 Sep 19	8		
				Wet	2015 Sep 20	5		
				Wet	2017 Aug 24	4		
7	Vientiane	Meungsong	Trade	Dry	2016 Mar 17	4	11 (3.1)	2 (18.2)
				Wet	2015 Aug 9	5		
				Wet	2017 Aug 25	2		
8	Vientiane	Pha-hom	Trade (roadside market)	Wet	2016 Jun 15	1	1 (0.3)	0/1 (0.0)
9	Xiengkhuang	Nam-ngam	Trade	Wet	2016 May 12	4	14 (3.9)	3 (21.4)
				Wet	2016 May 13	3		
				Wet	2016 May 16	6		
				Wet	2017 Aug 22	1		
10	Xiengkhuang	Phonsavanh/ Souanphukham	Trade	Dry	2014 Dec 18	2	34 (9.5)	6 (17.6)
				Dry	2016 Mar 11	9		
				Dry	2016 Mar 12	5		
				Dry	2016 Mar 13	5		
				Wet	2015 Sep 15	4		
				Wet	2015 Sep 16	4		
				Wet	2015 Sep 17	4		
				Wet	2016 May 15	1		
11	Xiengkhuang	Thajok	Trade (roadside stall)	Wet	2016 May 13	3	4 (1.1)	1 (25.0)
				Wet	2016 May 16	1		
BKX	Bolikhamxay	Bolikhamxay POFI	POFI	Dry	2014 Nov 13	4	28 (7.8)	22 (78.6)
				Dry	2015 Apr 23	4		
				Dry	2017 Jan 18	6		
				Wet	2014 Jul 10	2		
				Wet	2016 Jul 26	5		
				Wet	2016 Aug 29	5		
				Wet	2016 Aug 30	2		
CHP	Champasak	Champasak POFI	POFI	Wet	2017 May 19	1	1 (0.3)	1 (100.0)
VTE	Vientiane Capital	Vientiane Capital POFI	POFI	Wet	2016 Jul 7	6	6 (1.7)	2 (33.3)
TOTAL							359 (100.0)	92 (25.6)

*POFI, Provincial Office of Forestry Inspection.

Appendix Table 2. Sampling methods used in wildlife traded in markets for human consumption, Laos*

Sample type	Animal status	Method	Sample preservation
Urogenital swab	Live or dead	Urogenital area swabbed (Puritan Medical Products, https://www.puritanmedproducts.com), collected in duplicate.	VTM + RNAlater†
Urine	Live	Plastic sheet was placed under cages and left until the animal urinated (or for a maximum of 30 min). Collected urine was transferred to tube.	Plain tube
	Dead	Collected either by using a disposable pipette after pressing on the bladder or by cystocentesis using a 21G needle and sterile syringe. When insufficient urine was available, the urogenital area was swabbed up to 2 times.	Plain tube *or* VTM + RNAlater (swabs)
Blood	Dead	Blood drawn by rib cage cardiac puncture using a 21G needle	Plain tube
Liver	Dead (nonbutchered)	Obtained using a punch biopsy needle (Single Action Biopsy Device 14G, 20 mm Throw Trocar Tip, Argon Medical Devices, https://www.argonmedical.com).	VTM + RNAlater
Liver, kidney, or spleen	Dead (butchered or collected by POFI)	≈200 mg tissue samples collected	VTM + RNAlater

*POFI, Provincial Office of Forestry Inspection; VTM, viral transport medium (in-house formulation, National Animal Health Laboratories, Vientiane, Laos).
†RNAlater (Sigma-Aldrich, https://www.sigmaaldrich.com).

Appendix Table 3. PCR assays used for pathogen detection in wildlife traded in markets for human consumption, Laos*

Pathogen	Name	Sequence 5′-3′	Target region	Reference
O. tsutsugamushi	OtsuFP630	AACTGATTTTATTCAAACTAATGCTGCT	47 kDa outer membrane protein	(*4*)
	OtsuRP747	TATGCCTGAGTAAGATACRTGAATRGAATT		
	OtsuPR665	FAM-TGGGTAGCTTTGGTGGACCGATGTTTAATCT-TAMRA		
Rickettsia spp.	R17K128F2	GGGCGGTATGAAYAAACAAG	17 kDa surface antigen	(*4*)
	R17K238R	CCTACACCTACTCCVACAAG		
	R17K202TAQP	FAM-CCGAATTGAGAACCAAGTAATGC-TAMRA		
Nested *Rickettsia* spp.	R17kM61F	ACTTTACAAAATTCTAAAAACCATATACT	17 kDa surface antigen	(*5*)
	R17K31F	GCTCTTGCAGCTTCTATGTTACA		
	Rr2608Rnew	CATTGTCCGTCAGGTTGGCG		
Leptospira spp.	Lepto-F	CCCGCGTCCGATTAG	16s rRNA	(*6*–*8*)
	Lepto-R	TCCATTGTGGCCGRACAC		
	Lepto-probe	FAM-CTCACCAAGGCGACGATCGGTAGC-BHQ1		
Anaplasmataceae (*Neorickettsia* spp./*Anaplasma* spp./*Ehrlichia* spp.)	Ehr-16S_F	GGTACCYACAGAAGAAGTCC	16s rRNA	(*9*)
	Ehr-16S_R	TAGCACTCATCGTTTACAGC		
E. chaffeensis	ECH16S-17	GCGGCAAGCCTAACACAT	16s rRNA	(*10*)
	ECH16S-97	CCCGTCTGCCACTAACAATTATT		
	ECH16S-38	carboxyfluorescein-AGTCGAACGGACAATTGCTTATAACCTTTTGGT		
A. phagocytophilum	ApMSP2f	ATCGAAGGTAGTGTTGGTTATGGTATT	msp2 outer membrane protein	(*11*)
	APMSP2r	TTGGTCTTGAAGCGCTCGTA		
	APMAP2p	HEX-TGGTGCCAGGGTTGAGCTTGAGATTG-TAMRA		
C. burnetii	IS1111f	CAAGAAACGTATCGCTGTGGC	IS1111 transposase	(*12*)
	IS1111R	CACAGAGCCACCGTATGAATC		
	IS1111 probe	FAM-CCGAGTTCGAAACAATGAGGGCTG-TAMRA		
Flavivirus	PF1	TGYRTBTAYAACATGATGGG	NS5	(*13*)
	PF2bis	GTGTCCCAICCNGCNGTRTC		
	PF3	ATHTGGTWYATGTGGYTDGG		
Dengue virus	DenAll-F	AGGACYAGAGGTTAGAGGAGA	3′UTR	(*14*)
	DenAll-R	CGYTCTGTGCCTGGAWTGAT		
	DenAll-P	FAM-ACAGCATATTGACGCTGGGARAGACC-TAMRA		
Hantavirus	PanHanta-F2	TGCWGATGCIACRAAATGGTC	L segments	(*15*)
	PanHanta-R2	GCATCATCWGARTGATGIGCAA		
Zika virus	ZIKA2 S	CTTGGAGTGCTTGTGATT	Polyprotein	†
	ZIKA2 R	CTCCTCCAGTGTTCATTT		
	ZIKA2 PROBE	FAM-AGAAGAGAATGACCACAAAGATCA-TAMRA		
Universal	27F/V1-F	AGAGTTTGATCMTGGCTCAG	16S rRNA	(*16, 17*)
	518R/V3-R	GTATTACCGCGGCTGCTGGCA		
T4	T4F	CCATCCATAGAGAAAATATCAGAACGA	Enterobacteria phage T4	(*18*)
	T4R	TAAATAATTCCTCTTTTCCCAGCG		
	T4probe	VIC-AACCAGTAATTTCATCTGCTTCTGATGTGAGGC-TAMRA		
MS2	MS2F	CTCTGAGAGCGGCTCTATTGGT	Enterobacteria phage MS2	(*18*)
	MS2R	GTTCCCTACAACGAGCCTAAATTC		
	MS2probe	VIC-TCAGACACGCGGTCCGCTATAACGA-TAMRA		

*Assays used 5 µL of DNA/RNA template with a 20 µL PCR mastermix containing 0.8 µg/µL BSA. Bacterial quantitative PCRs used Platinum Quantitative PCR SuperMix-UDG (ThermoFisher Scientific, https://www.thermofisher.com), while either SuperScript III Platinum One-Step qRT-PCR system (ThermoFisher Scientific) or QuantiTect SybrGreen quantitative reverse transcription PCR kit (QIAGEN, https://www.giagen.com) was used for viral quantitative reverse transcription PCR. A cycle threshold value of <40 was classified as positive. Where applicable, primers were also used for sequencing of PCR products.

†Inhouse quantitative reverse transcription PCR by Unité des Virus Emergents, L'Institut de recherche pour le développement, Faculté de Médecine, Timone, Marseille, France.

Appendix Table 4. Number and type of animal species sampled in wildlife markets, Laos

Order	Family	Scientific name	Common name	No. (%)
Artiodactyla	Cervidae	*Muntiacus muntjak*	Red muntjac	1 (0.3)
Carnivora	Felidae	*Catopuma temminckii*	Asian golden cat	1 (0.3)
		Prionailurus bengalensis	Leopard cat	3 (0.8)
	Herpestidae	*Herpestes javanicus*	Small Asian mongoose	3 (0.8)
	Mustelidae	*Martes flavigula*	Yellow-throated marten	1 (0.3)
	Viverridae	*Arctogalidia trivirgata*	Small-toothed palm civet	2 (0.6)
		Paguma larvata	Masked palm civet	2 (0.6)
		Paradoxurus hermaphroditus	Common palm civet	24 (6.7)
		Viverra megaspila	Large-spotted civet	1 (0.3)
		Viverricula indica	Small Indian civet	1 (0.3)
Chiroptera	Pteropodidae	*Cynopterus* sp.	Bat sp.	3 (0.8)
		Eonycteris spelaea	Cave nectar bat	3 (0.8)
		Macroglossus sobrinus	Greater Long-tongued nectar bat	1 (0.3)
		Megaerops sp.	Bat sp.	2 (0.6)
		Rousettus sp.	Bat sp.	14 (3.9)
		Sphaerius sp.	Bat sp.	2 (0.6)
Lagomorpha	Leporidae	*Lepus peguensis*	Burmese hare	1 (0.3)
Rodentia	Hystricidae	*Atherurus macrourus*	Brush-tailed porcupine	1 (0.3)
	Muridae	Unknown	Rat sp.	1 (0.3)
	Sciuridae	*Belomys pearsonii*	Hairy-footed flying squirrel	12 (3.3)
		Callosciurus erythraeus	Pallas's squirrel	73 (20.3)
		Callosciurus finlaysonii	Variable squirrel	29 (8.1)
		Callosciurus inornatus	Inornate squirrel	40 (11.1)
		Dremomys rufigenis	Red-cheeked squirrel	36 (10.0)
		Hylopetes alboniger	Particolored flying squirrel	5 (1.4)
		Hylopetes phayrei	Phayre's flying squirrel	9 (2.5)
		Hylopetes sp.	Small flying squirrel	4 (1.1)
		Hylopetes spadiceus	Red-cheeked flying squirrel	2 (0.6)
		Menetes berdmorei	Indochinese ground squirrel	29 (8.1)
		Petaurista elegans	Lesser giant flying squirrel	1 (0.3)
		Petaurista petaurista	Red giant flying squirrel	2 (0.6)
		Petaurista philippensis	Indian giant flying squirrel	14 (3.9)
		Petaurista sp.	Giant flying squirrel	1 (0.3)
		Ratufa bicolor	Black giant squirrel	3 (0.8)
		Unknown	Small flying squirrel	2 (0.6)
	Spalacidae	*Rhizomys pruinosus*	Hoary bamboo rat	21 (5.8)
		Rhizomys sumatrensis	Indomalayan bamboo rat	6 (1.7)
Scandentia	Tupaiidae	*Tupaia belangeri*	Northern treeshrew	3 (0.8)
Total				359

Appendix Table 5. Wildlife specimens collected in markets and results of PCR tests for zoonotic pathogens, Laos*

		No. positive/No. tested										
Animal specimens from trade sites	No.	16S Universal cPCR	*Leptospira* spp. q-PCR	*Coxiella* q-PCR	*Anaplasma phagocytophilum* q-PCR	*Ehrlichia chaffeensis* q-PCR	*Orientia tsutsugamushi* q-PCR	Anaplasmatacae cPCR	*Rickettsia* spp. qPCR	*Rickettsia typhi* qPCR	Dengue virus qRT-PCR	Flavivirus qRT-PCR	Zika virus qRT-PCR
Arctogalidia trivirgata	2	–	2/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Atherurus macrourus	1	–	1/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Belomys pearsonii	12	–	1/12	n.s	n.s	n.s	n.s	n.s	n.s	–	0/12	0/12	0/12
Callosciurus erythraeus	56	–	8/56	0/9	0/9	0/9	0/9	0/9	0/8	–	0/56	0/56	0/55
Callosciurus finlaysonii	28	–	13/28	0/2	0/2	0/2	0/2	0/2	0/2	–	0/28	0/28	0/28
Callosciurus inornatus	34	–	7/34	0/3	0/3	0/3	0/3	0/3	0/3	–	0/34	0/34	0/34
Cynopterus spp.	3	–	0/3	n.s	n.s	n.s	n.s	n.s	n.s	–	0/3	0/3	0/3
Dremomys rufigenis	35	–	5/35	0/11	0/11	0/11	0/11	0/11	2/11	0/1	0/35	0/35	0/35
Eonycteris spelaea	3	–	1/3	0/2	0/2	0/2	0/2	0/2	0/2	–	0/3	0/3	0/3
Herpestes javanicus	3	–	0/3	0/2	0/2	0/2	0/2	0/2	0/2	–	0/3	0/3	0/3
Hylopetes alboniger	5	–	1/5	n.s	n.s	n.s	n.s	n.s	n.s	–	0/5	0/5	0/5
Hylopetes phayrei	9	–	1/9	n.s	n.s	n.s	n.s	n.s	n.s	–	0/9	0/9	0/9
Hylopetes spp.	4	–	0/4	n.s	n.s	n.s	n.s	n.s	n.s	–	0/4	0/4	0/4
Hylopetes spadiceus	2	–	1/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Lepus peguensis	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Macroglossus sobrinus	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Martes flavigula	1	–	0/1	0/1	0/1	0/1	0/1	0/1	0/1	–	0/1	0/1	0/1
Megaerops spp.	2	–	0/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Menetes berdmorei	29	–	4/29	0/1	0/1	0/1	0/1	0/1	0/1	–	0/29	0/29	0/29
Muntiacus muntjak	1	–	1/1	0/1	0/1	0/1	0/1	1/1	0/1	–	0/1	0/1	0/1
Paguma larvata	2	–	1/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Paradoxurus hermaphroditus	22	–	10/22	0/6	0/6	0/6	0/6	1/6	1/6	0/1	0/22	0/22	0/22
Petaurista elegans	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Petaurista petaurista	2	–	0/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Petaurista philippensis	9	–	1/9	0/2	0/2	0/2	0/2	0/2	1/2	–	0/9	0/9	0/9
Petaurista spp.	1	–	0/1	0/1	0/1	0/1	0/1	0/1	0/1	–	0/1	0/1	0/1
Prionailurus bengalensis	3	–	1/3	n.s	n.s	n.s	n.s	n.s	n.s	–	0/3	0/3	0/3
Ratufa bicolor	2	–	0/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Rhizomys pruinosus	21	–	3/21	n.s	n.s	n.s	n.s	n.s	n.s	–	0/21	0/21	0/21
Rhizomys sumatrensis	6	–	1/6	n.s	n.s	n.s	n.s	n.s	n.s	–	0/6	0/6	0/6
Rousettus spp.	14	–	0/14	n.s	n.s	n.s	n.s	n.s	n.s	–	0/14	0/14	0/13
Sphaerius spp.	2	–	0/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Tupaia belangeri	3	–	1/3	n.s	n.s	n.s	n.s	n.s	n.s	–	0/3	0/3	0/3
Unknown Sciuridae	2	–	1/2	n.s	n.s	n.s	n.s	n.s	n.s	–	0/2	0/2	0/2
Viverra megaspila	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Viverricula indica	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1
Animal specimens from POFI													
Callosciurus erythraeus	17	2/2	4/17	0/17	0/17	0/17	0/17	0/17	11/17	6/11	0/17	0/17	0/16
Callosciurus finlaysonii	1	–	1/1	0/1	0/1	0/1	0/1	0/1	0/1	–	0/1	0/1	0/1
Callosciurus inornatus	6	–	2/6	0/6	0/6	0/6	0/6	0/6	2/6	0/2	0/6	0/6	0/6
Catopuma temminckii	1	–	1/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1
Dremomys rufigenis	1	–	0/1	0/1	0/1	0/1	0/1	0/1	0/1	–	0/1	0/1	0/1
Paradoxurus hermaphroditus	2	–	0/2	0/2	0/2	0/2	0/2	0/2	1/2	–	0/2	0/2	0/2
Petaurista philippensis	5	–	1/5	0/4	0/4	0/4	0/4	0/4	3/5	0/2	0/5	0/5	0/5
Ratufa bicolor	1	–	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1

Animal specimens from trade sites	No.	No. positive/No. tested										
		16S Universal cPCR	*Leptospira* spp. q-PCR	*Coxiella* q-PCR	*Anaplasma phagocytophilum* q-PCR	*Ehrlichia chaffeensis* q-PCR	*Orientia tsutsugamushi* q-PCR	Anaplasmatacae cPCR	*Rickettsia* spp. qPCR	*Rickettsia typhi* qPCR	Dengue virus qRT-PCR	Flavivirus qRT-PCR	Zika virus qRT-PCR
Unknown Muridae	1	–	0/1	0/1	0/1	0/1	0/1	0/1	0/1	–	0/1	0/1	0/1
Sample types taken from trade sites													
Blood	85	–	9/85	0/1	0/1	0/1	0/1	0/1	0/1	–	0/85	0/85	0/85
Kidney	6	–	2/6	0/6	0/6	0/6	0/6	4/6	0/6	–	0/6	0/6	0/6
Liver	40	–	1/40	0/40	0/40	0/40	0/40	3/40	0/40	0/2	0/40	0/40	0/40
Spleen	3	–	1/3	0/3	0/3	0/3	0/3	1/3	0/3	–	0/3	0/3	0/3
Urine	15	–	1/15	n.s	n.s	n.s	n.s	n.s	n.s	–	0/15	0/15	0/14
Urogenital swab	312	–	58/312	0/18	0/18	0/18	0/18	0/18	0/18	–	0/312	0/312	0/311
Sample types from POFI-collected animals													
Blood	3	–	1/3	n.s	n.s	n.s	n.s	n.s	n.s	–	0/3	0/3	0/3
Kidney	91	–	14/91	0/91	0/91	0/91	0/91	5/91	29/91	4/27	0/91	0/91	0/90
Liver	92	1/1	14/92	0/92	0/92	0/92	0/92	4/92	34/92	2/29	0/92	0/92	0/92
Spleen	69	1/1	17/69	0/69	0/69	0/69	2/69	8/69	17/69	1/17	0/69	0/69	0/69
Urogenital swab	1	–	0/1	n.s	n.s	n.s	n.s	n.s	n.s	–	0/1	0/1	0/1

*cPCR, conventional PCR; n.s., no appropriate sample available; POFI, Provincial Office of Forestry Inspection; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcription PCR; –, not tested;

Appendix Table 6. Descriptions of organisms identified in wildlife traded for human consumption, Laos

Organism	Human pathogen	Notes	Reference
Rickettsia typhi	Yes	Cause of murine typhus, a major underrecognized cause of fever in Laos	(*19*)
Rickettsia felis	Yes	An emerging rickettsial pathogen, often misdiagnosed as other febrile illnesses	(*20*)
Orientia tsutsugamushi	Yes	Cause of scrub typhus, responsible for up to 23% of fever cases in Laos. Vectors are *Leptotrombidium* mites. Commonly associated with ground-dwelling rodents, but vectors are known to parasitize squirrels. *O. tsutsugamushi* has been isolated from *Callosciurus notatus* (plantain squirrel) in Malaysia	(*21*–*23*)
Anaplasma phagocytophilum	Yes	Cause of human granulocytic anaplasmosis	(*24*)
Anaplasma platys	Yes	Been identified in those with close associations with infected animals, such as veterinarians and companion animal owners	(*25*)
Anaplasma capra	Yes	Has been identified in humans following tick bites	(*26*)
Anaplasma marginale	No	Closely related to *A. centrale*. Known cause of bovine anaplasmosis	(*27*)
Anaplasma centrale	No	A known cause of bovine anaplasmosis	(*27*)
Anaplasma bovis	No	A known cause of bovine anaplasmosis. Not reported to infect humans	(*24*,*28*,*29*)
Ehrlichia chaffeensis	Yes	Cause of human monocytic ehrlichiosis	(*30*)
Lactococcus garvieae	Rare	Rare cause of human opportunistic infections	(*31*)
Kurthia species	Rare	Rare cause of human opportunistic infections	(*32*)